

April 27, 1882.

THE PRESIDENT in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The Right Hon. Lord Bramwell and Dr. Ramsay H. Traquair were admitted into the Society.

The following Papers were read :—

I. "On the Attitudes of Animals in Motion." By EADWEARD MUYBRIDGE, of Palo Alto, California. Communicated by THE PRESIDENT. Received April 20, 1882.

II. "Preliminary Account of the Structure of the Cells of the Liver, and the Changes which take place in them under Various Conditions." By J. N. LANGLEY, M.A., Fellow of Trinity College, Cambridge. Communicated by Dr. MICHAEL FOSTER, Sec. R.S. Received April 24, 1882.

I have examined the structure of the liver-cells in the frog, toad, newt, the common snake, the grey lizard, the roach and smelt, the pigeon, and various mammals. In all these the "resting" liver cells have the following common points of structure.

The protoplasm is arranged in the form of a network or honeycomb, the meshes or spaces of which are in all parts of the cell of much the same size; the outer parts of the cell are formed of a thin layer of slightly modified protoplasm with which, however, the network is continuous.

The spaces of the protoplasmic network are occupied by paraplasm or interfibrillar substance, consisting of (1) spherical granules, probably proteid in nature; (2) spherical globules of fat; (3) hyaline substance, filling up the spaces not occupied by the granules or globules. This substance consists partly of glycogen and in part probably of a proteid.

This description differs in several points from those given by previous observers. The account given by Klein* resembles it more

* Klein, "Quart. Journ. Med. Sc.," N.S., xix, p. 161, 1879.

nearly than any other. Klein described the liver-cells of mammals as consisting of a protoplasmic network and of a hyaline interfibrillar substance; any apparent granules present he considered to be nodal points of the network. In preparations made after Klein's methods the granules are in fact with difficulty, or not at all, to be made out. They are, however, very obvious in fresh teased or in osmic acid specimens, especially in such specimens of the liver of the mole.

Previous to Klein's account of the mammalian liver-cells, Kupffer* described the liver-cells of the frog as consisting of a protoplasmic network and of hyaline paraplastm. He figures the network as being very irregular, with small granules in its bars, as being completely absent from considerable portions of the cell, and as having much finer meshes in the outer part of the cell around the nucleus. Kupffer's observations were made upon the tissue treated with osmic acid or with 10 per cent. salt solution and iodine. In sections of a frog's liver hardened in osmic acid I find the cells to consist of a slightly stained mass, in which granules and fat globules are imbedded. Treatment with other reagents shows that this slightly stained mass really consists of a network, and interfibrillar substance, the latter not in a granular form, but occupying the spaces in the network unoccupied by the granules and globules. If the liver is exposed for some time before it is placed in osmic acid, the granules are no longer distinct; an irregular network-like structure is formed out of them. This may possibly be the network of Kupffer. Further, in the liver-cells of a healthy, hungry (not fasting) frog the protoplasmic network stretches fairly equally throughout all parts of the cell; in winter frogs which have long fasted (cf. below, p. 22) the network has wider spaces and thinner bars in one part of the cell than in the other; but then the wider meshes are found in the outer, and not in the inner part of the cell and are not absent from any cell region. Lastly, in osmic acid specimens of such cells the outer parts appear homogeneous, except for fat globules, the inner zone is crowded with distinct granules.

Hence it is obvious that the network of protoplasm and the paraplastm described by Kupffer do not in the least correspond to the network of protoplasm and interfibrillar substance described by myself. Whilst the liver-cells of all classes of vertebrates which I have examined have the above-mentioned common characters in the "resting" state, they have certain minor distinguishing characters in each class, depending chiefly upon the size of the cells and their nuclei, the position of the nuclei, and the relative amount of the various cell-constituents present. Of these, however, I do not propose to give an account here.

Hering, in 1867, pointed out that the liver in all vertebrates except

* Kupffer, "Festschrift an Carl Ludwig," 1875.

mammals is an anastomosing tubular gland. Hence we should expect that the changes which take place during digestion in the liver of an amphibian, reptile, fish, or bird should differ somewhat from those which take place during digestion in the liver of a mammal. I have taken the liver of the frog and toad as an example of the tubular gland form, and have made observations on this during the last two years and a half. How nearly the changes which take place in other "tubular" livers resemble those which take place in the "tubular" livers of the frog and toad can of course only be decided by direct observation; but it seems unlikely that the change should take any very different course, since the resting state of the cells and their arrangement in the gland is so very nearly the same.

The Liver of the Frog.

The liver of the frog and that of the newt can be observed while the blood continues to circulate through it; in this state nothing is seen in the cells except the fat globules; if a small piece be teased out in salt solution, the faintly outlined granules are seen floating in the fluid; these become obvious on adding iodine. The best method for bringing out the granules and fat globules is to place a small piece of the liver in osmic acid, 1 per cent., for a day, to transfer it to alcohol for several days, and then to prepare sections; in such sections the granules and globules appear to be imbedded in homogeneous cell substance. The granules are largest in the liver of the newt, so that the liver of this animal is best adapted to show that the granules are not the nodal points of the network. The network of the cell is brought out most distinctly by chromic acid, 0.5 per cent.; it can also be seen in specimens hardened in picric acid, alcohol, mercuric chloride, or in teased out specimens of a liver which has been treated with neutral ammonium chromate, 5 per cent.

When a section of the liver which contains glycogen, and which has been hardened in alcohol or osmic acid, is placed for some minutes in iodine solution, certain parts of the interfibrillar substance of the liver-cells stain red-brown. The substance which is so stained I conclude to be glycogen. By water, even at 30° C., it is only slowly dissolved. Since isolated glycogen is readily soluble in water, this might lead us to think that the red-staining substance is not glycogen; but we know that glycogen is not readily extracted from the liver by warm water, and hence it seems probable that a large part of the glycogen of the liver exists in it in a form not very soluble in water; but, whatever may be the condition of glycogen in the liver, I think I am justified in concluding that the red-staining substance in alcohol specimens is glycogen, since glycogen can be extracted from them, since the amount of the red-staining substance varies directly with the amount of glycogen which can be extracted, since the coloration

is just that produced when iodine is added to a little purified glycogen on a glass slide, and since it is rapidly dissolved by amylolytic ferment, such as an aqueous or glycerine extract of the parotid of a rabbit.

During the summer months the liver of a hungry frog has granules scattered equally throughout the cell, and there is very little glycogen. During the long winter fast the cells change in appearance; the granules become more and more confined to the inner part of the cell, and form there a marked inner granular zone. The glycogen increases in amount, and is stored up chiefly in the outer part of the cell, where there are no granules. Osmic acid specimens of glands in this condition show two distinct zones, an inner granular one and an outer, apparently homogeneous, one; the nucleus lies at the border of the two, or if the outer zone is large lies in it alone. When such a specimen is treated with iodine, all or nearly all of the outer zone stains red-brown; around the granules also a varying amount of red-brown stained substance is found; the network of the inner zone, the granules, and the nucleus stain yellow. In these specimens the network of the outer part of the cell cannot at all, or only very imperfectly be made out. It is, however, seen in sections of the gland which have been hardened in chromic acid. It is continuous with the network of the inner part of the cell, but has wider spaces, and its bars are finer.

We know that in the œsophageal glands of the frog and in such gastric glands as have been investigated on the point, the changes which take place in the cells in fasting closely resemble the changes which take place in them during digestion. So here, in the liver of the frog, the changes which take place when the animal is fed closely resemble those gradually established during the winter fasting period. The extent of the changes occurring in digestion depends greatly upon the state of the liver cells before the animal is fed; in summer the changes are slight, there is only a slight decrease of granules in the outer part of the cell and a slight increase of glycogen. The changes are much more marked when the cells have to start with a small outer non-granular zone; in such cases in the 6th to 8th hour of digestion the outer zone is large, and in the 24th to 30th the cells become granular throughout. When a frog which has already a large outer non-granular zone is fed the decrease of granules usually lasts a shorter time, and in the 6th to 8th hour of digestion the granules begin to increase. In other words, the using up and formation of granules go on at different relative rates in different nutritive conditions of the body.

The disappearance of granules and the formation of glycogen which takes place in winter frogs is only partly brought about by the absence of food; it is brought about in part perhaps chiefly by the low temperature. If winter frogs, the liver-cells of which have few

granules and much glycogen, are kept at about 20° C. for a week to a fortnight, the cells become granular throughout and the glycogen largely disappears; similarly frogs in spring or autumn, the liver-cells of which have many granules and little glycogen, if kept at a low temperature a week to a fortnight, present in the cells of the liver an outer non-granular zone and an increase of glycogen; in summer frogs the effect is much less. Further, the changes during digestion are slight in winter frogs that have been kept in the warm, greater in spring and autumn frogs which have been kept in the cold.

Although, generally speaking, a decrease of granules goes hand-in-hand with an increase of glycogen and an increase of granules with a decrease of glycogen, yet a certain amount of variation in the one may take place without any variation or any corresponding variation in the other. Hence I regard the formation of granules and the formation of glycogen as independent processes. A comparison of the changes which take place in the granules of the liver-cells with the changes which take place in the granules of the salivary, gastric, and pancreatic glands leaves me with no doubt that the granules of the liver-cells are destined to give rise to some constituent or constituents of the bile, and it seems to me more than probable that by appropriate chemical treatment, we may obtain from them some one or more of the constituents of the bile-salts.

In the account I have just given I have omitted all mention of the fat globules. These vary so much with different conditions of the body, as yet unknown, that it is extremely difficult to determine what changes in their number and position take place during digestion. It is well known that there is in winter an increase in the amount of fat in the liver. Generally speaking, in summer the fat globules are small, few, and fairly equally scattered throughout the cell, with a tendency to be more numerous around the lumen. In winter frogs the greatest variation occurs, occasionally there are very few, usually there are a considerable number, not unfrequently the cells are crowded with them, this is generally the case with obviously unhealthy frogs. Further, in the most common condition, that in which the fat globules are fairly numerous without being crowded together in the cells, they may occur almost entirely in the inner or almost entirely in the outer part of the cell. In the former case they make a conspicuous fat globule zone about the lumen, in the latter they occur in conspicuous clumps close to the outer cell-border; this is generally the case when one has reason to suppose that fat is increasing in the cells. If winter frogs are kept in the warm, the fat globules diminish in number; if summer frogs are kept in the cold for a week to a fortnight there is a slight, but only a slight, increase in the quantity of fat. The majority of frogs which are fed in the summer show little or no change in the number or size of fat globules in the liver.

In winter the amount of fat in the liver varies so much in frogs apparently alike, that I do not feel justified in drawing any conclusions as to the changes occurring in digestion.

The only other vertebrates with liver of the tubular type on which I have made a series of observations, are the toad and newt. The changes in the liver of the toad are in all essential respects the same as those I have described for the liver of the frog. In the newt's liver I have not observed the formation of zones during digestion; it appears to me to depart largely from the tubular type of gland, and to resemble in structure rather the mammalian than the ordinary amphibian liver. In the snake during the winter's fast, an outer non-granular zone makes its appearance in the liver tubules; it is, however, much smaller than that in the frog and toad.

I may mention that the cells of the frog's bile-duct, where it runs through the pancreas, are ciliated; the pancreatic duct with conciliated cells joins it close to the small intestine.

The Mammalian Liver.

In the mole the granules of the liver are conspicuous, and are preserved by osmic acid; in the dog, cat, and rabbit the granules are more or less altered, hence I have chosen the mole to make observations upon as to the changes which take place in digestion. In the hungry animal the protoplasmic network stretches throughout the cells with nearly equally sized meshes; in the spaces of the network is a small amount of hyaline substance, partly glycogen, and embedded in this are rather large granules. When the liver is examined six to eight hours after digestion, there is a greater or less disappearance of granules from the centre of the cell around the nucleus; the network here has wider spaces and thinner bars, and the spaces are for the most part filled with glycogen. In cases where these changes are most marked, osmic acid specimens treated with iodine show a diffuse reddish stained mass surrounding the nucleus; at the borders of the cell, the yellow stained network is seen, and one or two rows of granules; between these a little red stained substance may usually be traced continuous with the central mass of glycogen; the peripheral network and granules make the cell appear at first sight as if it had a very thick cell wall. The network in the central part of the cell is brought out by hardening a piece of the liver in chromic acid and other reagents. In cells in which the digestive changes are less advanced, the glycogen may only partially surround the nucleus, or may be accumulated more on one side of it than elsewhere.

Histological observations on the increase of glycogen in the liver-cells have been made by Bock and Hofmann,* on the rabbit, and by

* Bock u. Hofmann, "Virchow's Archiv.," 56, s. 201, 1872.

Kayser,* on the dog. Bock and Hofmann found that glycogen accumulated in amorphous form around the nucleus, and stretching out from this as a network towards the periphery of the cell. My own observations confirm this in the main. I regard, however, the glycogen as being stored up in the spaces of a protoplasmic network. Bock and Hofmann also mention granules of the liver-cells which stain yellow with iodine,† but they do not appear to have distinguished between these and the protoplasmic network which likewise stains yellow with iodine.

Kayser (*loc. cit.*) found that glycogen was stored up in the form of "Schollen oder Körner;" these lumps and granules of glycogen I have not seen; an appearance simulating this occurs when the glycogen is stored up more in some parts of the protoplasmic network around the nucleus than in others, but these local collections never have, so far as I have observed, sharply marked boundaries. The granules are not distinguished by Kayser from the protoplasmic network; these two together make up, I imagine, the thick peripheral layer of protoplasm described by him as occurring in cells which contain much glycogen. As in the liver of the frog, so in the mammalian liver, I take the granules to be concerned in the formation of some of the substances found in the bile.

* Kayser. Quoted by Heidenhain. "Hermann's Hdb.," Bd. v, Th. 1, s. 221, 1880.

† Similar granules were also described by Plosz. "Pflüger's Archiv.," vii, s. 371, 1873.